The Use of Peat Extracts/Products as a Feed Stock for Chemical and Biochemical Processes

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THE USE OF PEAT EXTRACTS/PRESSATE AS A FEED STOCK

FOR

CHEMICAL AND BIOCHEMICAL PROCESSES

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EXECUTIVE SUMMARY

This investigation was originally undertaken to examine the possibility of utilizing the soluble carbon compounds present in the large volumes of effluent from peat combustion processes such as wet carbonization, to obtain useful by-products by fermentation with Clostridium species. Within the first month of this sixth month study it was found that such extracts from fuel peat contain too low a concentration of fermentable substances to support any substantial microbial fermentation with these organisms. Therefore, with the agreement of the scientific authority, extracts of horticultural peat were obtained by controlled high pressure steam treatment using the Stake Technology process. It was predictable that some of the peat carbohydrate present in horticultural peat as hemicellulose and cellulose, would be solubilized by analogy with wood and other lignocellulosic matter which can be fractionated into hemicellulose, cellulose and lignin components using the Stake process. The investigation was considered worthwhile since fractionation of peat in this way could be used as the basis of a scheme for the total utilization of peat for chemical production, in which it would be necessary to obtain products of economic value from all the components.

Using the Stake process under conditions found to release the maximum amount of carbohydrate, a complex mixture of sugars utilizable by very few classes of microorganism was present in the peat extracts. However strains of clostridia are able to metabolize such complex substrates, and investigation showed that several strains which produce butanol, ethanol and acetone by fermentation, were able to grow well with the peat extract as sole carbon source. In a preliminary investigation of butanol production by C. acetobutylicum and C. butylicum, and ethanol production by C. thermohydrosulfuricum

using the small amounts of peat extract available, substrate utilization and solvent production were low, but these values could be increased in future investigations conducted on a larger scale in which greater control of the fermentation process would be possible. Such work would be necessary in order to judge the potential value of these fermentations as part of an industrial program utilizing all the components of peat in economically viable ways. In contrast to results obtained with extracts of wood from steam explosion treatment, fermentation was not inhibited in the peat extracts by the presence of toxic substances, which would be a major advantage when establishing an industrial fermentation process.

The peat residue after the steam explosion treatment contained a considerable amount of cellulose not available to cellulolytic bacteria. This would become available for chemical use, or for use as a substrate for many fermentation processes when hydrolysed to glucose, if the residue were treated to remove waxes, lignin and humic acid fractions for other useful applications. It may also be possible to solubilize more cellulose from peat by the addition of acid in the Stake steam explosion process.

The use of peat for chemical production by extraction or by fermentation frequently suggested but applied on a very limited scale in the past, may become a technically and economically feasible option by the application of modern technology. Previous reports on fermentation of peat have not been based on detailed knowledge of substrate composition, informed selection of the microorganism or consideration of other factors which affect the success of fermentation processess. The work described in this report addresses some of these factors in the use of peat extracts for solvent production by fermentation.

RÉSUMÉ ADMINISTRATIF

À l'origine, l'étude a été entreprise pour examiner la possibilité d'utiliser les composés carbonés solubles contenus dans les effluents volumineux provenant des procédés de combustion de la tourbe, telle la carbonisation humide, pour obtenir des sous-produits utiles après fermentation par des espèces de Clostridium. Dés le premier mois de cette étude qui devait en durer six, on a constaté que la concentration des substances fermentescibles dans ces extraits de tourbe combustible était trop faible pour permettre une fermentation microbienne valable par ces microorganismes. C'est pourquoi, avec l'accord du responsable scientifique du projet, on a obtenu des extraits de tourbe horticole par traitement contrôlé à la vapeur à haute pression, en utilisant le procédé de Stake Technology. On pouvait prévoir qu'une certaine partie des glucides présents dans la tourbe horticole sous forme de cellulose et d'hémicellulose se solubiliserait comme c'est le cas pour le bois et les autres produits lignocellulosiques qui peuvent être fractionnées en cellulose, en hémicellulose et en lignine à l'aide du procédé de Stake. Cette étude a été jugée utile, car un tel fractionnement de la tourbe pourrait servir de base à un système d'utilisation totale de la tourbe pour la production chimique, où il serait nécessaire de tirer de tous les constituants de la tourbe des produits ayant une valeur économique.

En appliquant le procédé Stake dans des conditions favorisant la libération d'une quantité maximale de glucides, on a obtenu à partir des extraits de tourbe un mélange complexe de sucres utilisables par un nombre très limité de classes de micro-organismes. Cependant, des souches de Clostridium sont capables de métaboliser ces subtrats complexes et une étude a révélé que plusieurs souches qui produisent du butanol, de l'éthanol et de

l'acétone par fermentation croissent très bien dans un milieu où l'extrait de tourbe constitue la seule source de carbone. Dans une étude préliminaire de la production de butanol par C. acetobutylicum et par C. butylicum et de la production d'éthanol par C. thermohydrosulfuricum à partir des petites quantités d'extrait de tourbe disponibles, on a constaté que l'utilisation du substrat et la production du solvant étaient faibles, mais qu'on pouvait accroître le rendement par d'autres travaux de recherche à plus grande échelle où il serait possible d'assurer une meilleure régulation du processus de fermentation. Un tel travail serait nécessaire pour juger de la valeur potentielle de ces fermentations dans le cadre d'un programme industriel visant à utiliser d'une manière économique tous les constituants de la tourbe. Contrairement aux résultats obtenus avec les extraits de bois traités par explosion à la vapeur, la fermentation des extraits de tourbe ne semble pas inhibée par la présence de substances toxiques, ce qui constituerait un avantage très important lorsqu'il s'agit de mettre sur pied un procédé de fermentation industrielle.

Le résidu de tourbe après traitement par explosion à la vapeur contenait une quantité considérable de cellulose non accessible aux bactéries cellulolytiques. Ce résidu deviendrait accessible pour utilisation chimique ou pour servir de substrat dans divers processus de fermentation après hydrolyse en glucose si le résidu était traité de façon à enlever les cires, la lignine et l'acide humique pour d'autres applications utiles. Il peut aussi être possible de solubiliser encore plus de cellulose provenant de la tourbe par addition d'acide dans le procédé Stake d'explosion à la vapeur.

L'utilisation de la tourbe pour l'obtention de produits chimiques par extraction ou par fermentation, souvent proposée dans le passé, mais appliquée uniquement à une échelle très limitée, pourrait devenir une activité réalisable, tant du point de vue

technique qu'économique, grâce à l'application de la technologie moderne. Les rapports antérieurs sur la fermentation de la tourbe n'ont pas été fondés sur une connaissance détaillée de la composition du substrat, sur une sélection éclairée du microorganisme fermenteur ou sur l'étude d'autres facteurs qui peuvent jouer un rôle dans le succès des processus de fermentation. Le présent rapport traite de certains de ces facteurs dans le cas de l'utilisation des extraits de tourbe pour produire des solvants par fermentation.

ABSTRACT

The Stake Technology steam explosion process, developed for the autohydrolysis of lignocellulosic materials, was used to treat peat containing 50% moisture. Treatment at 200°C for 3 minutes, followed by instantaneous decompression (steam explosion), solubilized 20 to 25% of the solids, and this material was largely carbohydrate. The sugars glucose, xylose, galactose, arabinose, and mannose accounted for 55% of the total carbohydrate in the soluble fraction, which was designated "peat extract". Galactose was the major sugar.

The peat extract was not toxic to Clostridium cultures at concentrations sufficient to provide 2% of carbohydrate. Higher concentrations were not tested because of a shortage of material. The peat extract carbohydrate was utilized by Clostridium acetobutylicum and Clostridium butylicum strains for the production of solvents. Between 30 and 40% of the carbohydrate was utilized for the production of fermentation products. Approximately 50% of the peat extract carbohydrate was also utilized by the thermophilic bacterium Clostridium thermohydrosulfuricum for growth and the production of ethanol.

The insoluble peat residue, from the steam explosion process, was treated with an acetic acid-nitric acid mixture to solubilize the non-cellulosic polymers. The residual cellulose accounted for 24% of this fraction. The untreated peat residue, when included in a culture medium, did not serve as a carbohydrate source for growth of the cellulolytic bacteria, Clostridium thermocellum and Bacteroides succinogenes.

INTRODUCTION

The purpose of this study was to examine the use of peat hydrolysates as alternate substrates in microbial fermentations of economic interest. The terms of reference of this particular project were confined to examination of the use of a dewatering by-product of fuel peat processing. Initial studies demonstrated that such material was unsuitable for microbial fermentations because the carbohydrate concentration was quite low, usually being less than 0.5%. Concentrations of 5.7% are generally thought to be the minimum required for commercial exploitation. Rather than continue with the project as planned, we, with the consensus of Dr. Frank Maine (Frank Maine Consulting) and Mr. John Sheppard (NRC), decided to focus our efforts on the use of undecomposed (or agricultural grade) peat as a fermentation substrate.

The use of agricultural grade peat presents another problem. In the absence of relatively severe chemical and/or physical pretreatments, there is little soluble carbohydrate in undegraded peat to support microbial growth. This is because much of the sugars are in polymeric form as cellulose, hemicellulose, or other macromolecules.

There are several pretreatment regimens available for the pretreatment of peat to release, or solubilize, carbohydrates. These processes have received adequate review elsewhere. Some, like the wet carbonization process, yield an effluent which is too dilute for fermentation purposes. Other technologies hold more promise. The Iotech and Stake Technology processes are two variants of a steam explosion treatment which can be adapted to peat processing. Because no dilution is involved in either process, the final carbohydrate concentration of the effluent is limited only by

condensation during processing relatively high concentrations of carbohydrate can therefore be obtained.

The first part of this research examined the effect of two processing parameters (time and temperature) on the release or solubilization of carbohydrates from peat treated by the Stake Technology Process. The optimal treatment, yielding a maximum of soluble saccharides, was identified, and a more detailed chemical analysis performed.

The second objective of this work was to examine the kinetics of growth and solvent production of selected bacterial strains of potential industrial application (see Table 1). Clostridium acetobutylicum and Clostridium butylicum are two solventogenic strains which have been used for the commerical production of butanol and acetone.

Other strains of Clostridium spp., such as C. thermohydrosulfuricum, C. thermocellum and C. thermosaccharolyticum, are the subject of much current interest for their potential in the production of ethanol (Ng et al. 1981; Murry and Asther, 1984; Zeikus, 1979; Zeikus et al. 1981). Previous studies suggest that these strains may be well suited to peat fermentations. Since they are thermophiles, there is less energy required for cooling of thermally-processed peat. In addition, C. thermohydrosulfuricum and C. thermosaccharolyticum possess the ability to utilize a wide range of substrates for growth, an important attribute of an organism being considered for the fermentation of a complex substrate like peat hydrolysate. C. thermocellum, a cellulolytic bacterium, was examined for its ability to metabolize peat residue, the insoluble by-product of the Stake Technology process. Bacteroides succinogenes, a cellulolytic rumen anaerobe, was similarily tested, in conjunction with C. thermocellum.

MATERIALS AND METHODS

(A) PRETREATMENT OF PEAT

(i) Steam treatment of peat samples

A 500g sample of horticultural peat (Acadia Peat Moss Ltd., Lameque, New Brunswick) was sieved through a 4 mm mesh screen and was given to Stake Technology, Ltd., (Oakville) to be thermally processed. Triplicate samples of peat (15g) were treated in triplicate for 0.5, 1.0, 2.0, 3.0, and 5.0 min at 170, 200, and 215°C. At the end of the treatment time, a ball valve was opened and the sample collected. Samples were stored at -20°C to await analysis.

Samples were separated into solid and supernatant fractions according to the scheme depicted in Figure 1. Dry weight, pH, and total carbohydrate released during processing were determined and the results tabulated (Table 4).

(ii) Preparation of additional hydrolysate by the Stake Technology process

On April 19, 1984, an additional 300 g of peat was processed at Stake Technology, to permit repetition of some of the earlier trials performed and to obtain sufficient quantities of substrate to conduct growth studies.

A quantity of peat (150 g) was treated at 200°C for 3.0 min. The hydrolysate from this treatment was used as medium substrate in growth studies. Approximately 25% of the total dry weight was unaccounted for in the soluble or insoluble fractions suggesting that considerable amounts of material are lost by volatilization and combustion during thermal processing.

(iii) **Analysis of peat hydrolysate provided by K. E. Chornet**

Approximately 2.0 l of peat hydrolysate, prepared by a wet carbonization process, was obtained from Dr K.E. Chornet of the University of Sherbrooke, P.Q. As supplied, the carbohydrate content of this hydrolysate was 0.08%, too low for fermentation trials without preliminary concentration. As a result, the investigation of this material was discontinued.

(B) ANALYSES OF PEAT HYDROLYSATES

Samples were prepared as described above (see A(i)). The hydrolysates were analysed for per cent solubilization of total material, for carbohydrate content (determined by the phenol-sulfuric acid method) and for reducing sugar (measured by the arsenomolybdate procedure) both before and after acid hydrolysis in 2.0 N HCl. The results of these analyses are listed in Table 5.

(i) **Carbohydrate determination**

Carbohydrate was determined essentially as described by Dubois et al. (1956). A 50 ul aliqout of 80% (wt/wt) phenol reagent was added to a 2.0 ml sample volume containing 0 to 75 ug of carbohydrate. After the addition of 5.0 ml concentrated sulfuric acid, the samples were thoroughly mixed and incubated at room temperature for a 1.0 h. Absorbance of the samples was determined at a wavelength of 490 nm using a Bausch and Lomb "Spectronic 20" spectrophotometer and compared to a glucose standard curve for quantification.

(ii) **Determination of reducing sugar**

Reducing sugar was determined by the arsenomolybdate method of Ashwell (1957). Samples (200 ul) containing 0 to 30 ug reducing sugar were mixed with 200 ul of Nelson-Somogyi reagent and heated at 100°C for 20 min. Arsenomolybdate reagent (200 ul) was added after cooling and the mixture

diluted to a final volume of 3 ml with distilled water. The tubes were centrifuged at 10,000xg for 5 min to remove sedimented insoluble material and the absorbance determined at a wavelength of 660 nm using a Gilford "Stasar II" spectrophotometer. Results are expressed as ug reducing sugar as glucose equivalents.

(iii) **Dry weight and ash determination**

To measure dry weight 15 mL samples of peat hydrolysate were placed into 20 mL vials and freeze dried in a Virtis "Unitrap II" lyophilizer. After recording the weight following desiccation the samples were ashed by combustion in a Gallenkamp muffle furnace (500°C, 24 h).

(iv) **Cellulose separation and estimation**

Cellulose was separated from associated polymers by extraction with an acetic-nitric reagent at 100°C for 30 min (Updegraff, 1969).

Samples (0.2g) of freeze dried peat were suspended in 6.0 ml of acetic acid-nitric acid reagent in 10 ml screw cap tubes and boiled for 30 min to solubilize any remaining hemicellulose. Samples (0.5 ml) were removed from the reaction volume and were washed three times with distilled water by centrifugation (16K x g; 5 min) in a table top centrifuge. Supernatants were discarded and the sediment resuspended to a volume of 1 ml with ice cold 67% (wt/vol) sulfuric acid. Samples were mixed thoroughly and left to stand for 60 min at room temperature. Aliquots were removed for total carbohydrate determination by the phenol-sulfuric acid method.

Cellulose recovery was measured by extracting 0.2 g samples of crystalline cellulose (Avicel PH 105) with acetic acid nitric acid reagent and comparing the results to those obtained with an unextracted cellulose standard. Recovery of

extracted cellulose was 100% in two separate experiments. Results are expressed as mg carbohydrate (as glucosyl residues) per gram dry weight.

(v) **Acid hydrolysis of peat extract and peat residues**

Both the peat residue and extract were hydrolysed under two conditions:

i) 140°C; 60 min in 0.6% (wt/vol) sulfuric acid
ii) 121°C; 120 min in 1.6% (wt/vol) sulfuric acid

For a review of acid solubilization of cellulosic materials see Grethlein (1978). The purpose of these treatments was to completely dissolve the polymeric materials found in the soluble and insoluble fractions. Carbohydrate and reducing sugar estimations were made to determine if the total available saccharides could be increased by these treatments. Reaction mixtures consisted of either 0.2 g solids (dry weight) or 1.5 mL of a peat extract containing 12% carbohydrate (wt/vol) were made to 5 mL with distilled water and either 31 or 81 ul of 98% (wt/vol) sulfuric acid added to yield a final acid concentration of 0.6% or 1.6%, respectively. The samples were placed in a 10 mL screw cap tube fitted with Teflon lined caps and treated as described above. In addition to carbohydrate and reducing sugar, the hydrolysates were also assayed for amino sugars and uronic acids.

(vi) **Determination of amino sugars**

Samples of acid hydrolysed (121°C, 2h, 1.6% sulfuric acid) peat extract and peat residue were assayed for hexosamine using the method of Rondle and Morgan (1955). Sulfate was removed by precipitation with an equi-molar amount of barium chloride. Results are expressed as mg amino sugar (as glucosamine) per gram dry weight.

(vii) **Determination of uronic acids**

The quantity of uronic acids in the peat extract was determined by the modified carbazole method of Davidson (1966). Results are expressed as mg uronic acids (as glucuronic acid) per gram dry weight.

(viii) **Determination of ammonia**

Ammonia was determined as described by Weatherburn (1967). Samples containing between 0 and 10 ug of ammonia nitrogen were made to a volume of 0.5 mL. Samples were mixed with 3 mL of alkaline phenolate reagent and 5 mL of sodium hypochlorite reagent. The assay mixtures were incubated for 90 min at room temperature after which the absorbance at 630 nm was determined in a Gilford "Stasar II" spectrophotometer. All samples were corrected for recovery of added ammonium sulfate standard.

(ix) **Determination of saccharide composition**

Aliquots (1.5 mL) of peat hydrolysate were centrifuged (16,000 x g, 5 min) and the supernatant (1 mL) eluted from a Pasteur pipette column containing equal amounts of Rexyn 201 (carbonate form) and Amberlite 112 - 120 (hydrogen form). The column was washed with 4 x 1 mL volumes of distilled water. The 5 mL sample volumes were then mixed and filtered through a 13 mm dia 0.45 u pore size Metricel membrane. Sample volumes (10 ul) were loaded onto the HPLC column using a Waters Model U6K injector.

Sugars were separated using a Biorad Aminex Ion-Exclusion HPX-87P column (300 x 7.8 mm) fitted with cationic and anionic deashing micro guard precolumns (Biorad Laboratories, Richmond, California). Samples (10 ul) were loaded onto the column were eluted at a flow rate of 0.6 mL min^{-1} and a temperature of 85°C using filtered, distilled water being the eluant. Eluted carbohydrate peaks were

monitored using a Model 7510 refractive index detector (Erma Optical Works, Tokyo, Japan). Peaks were recorded using a Sargent Welch SRM-2 log-linear recorder. Monosaccharide concentrations were calculated by reference to standard curves based on peak heights of the separated sugars.

(C) PRETREATMENT OF PEAT HYDROLYSATE SAMPLES FOR HIGH PRESSURE LIQUID CHROMATOGRAPHY

(i) Effect of sample preparation regimens on lignin removal from peat hydrolysates

To prevent a high back pressure and a deterioration in the separation with the Aminex Ion-Exclusion carbohydrate column it was necessary to remove ionic species, particulate material, and/or non-relevant biological material such as lignin, humic acids from all samples for HPLC analysis. Various resin and acid treatments were used to remove lignin and other materials. Lignin was monitored by measuring the change in absorbance, at 280 nm, in the soluble phase, of samples before and after acidification with sulfuric acid. Samples were then passed through a mixed bed resin [Rexyn 201 (carbonate form) - Amberlite IR-120 (hydrogen form)] or a hydrophobic resin (Amberlite XAD2) to determine if additional material could be removed by these treatments. HPLC analyses were performed simultaneously to ensure that the sugar composition of the peat hydrolysate did not change as a result of the treatment.

(ii) Binding of sugar standards to ion-exchange resins

The effect of ion-exchange pretreatment on sugar recovery was initially tested. Identical recoveries of the peat sugars were achieved regardless of whether samples were untreated, or passed through an Amberlite MB-3 mixed bed resin, an Amberlite IR-120 (hydrogen form)- Rexyn 201

(carbonate form, 16-50 mesh) mixed bed resin (Morita and Levesque, 1980), or an Amberlite IR-120 (hydrogen form)-Rexyn 201 (hydroxyl form, 16-50 mesh) resin. Samples were routinely deionized using the Amberlite IR-120 (hydrogen form)-Rexyn 201 (carbonate form) mixed bed resin prior to HPLC analysis.

(iii) **Binding of peat extract carbohydrate and reducing sugar to ion-exchange resins**

Pasteur pipette columns plugged with silanized glass wool were filled to a depth of 6 cm with various ion-exchange resins. The columns were then washed with glass distilled water to remove fines. A small volume (50 ul) of peat extract containing 12.0% carbohydrate (wt/vol) was added to the column and was eluted with distilled water to a volume of 5 ml. The collected fractions were assayed for both total carbohydrate by the phenol-sulfuric acid method and for reducing sugar by the arsenomolybdate assay.

Three types of mixed-bed resins were tested by the method described above:

i) Amberlite MB-3 - a commercial mixed bed resin.
ii) Rexyn 201 (carbonate form) + Amberlite IR-120 (hydrogen form)
iii) Rexyn 201 (hydroxyl form) + Amberlite IR-120 (hydrogen form)

Recoveries of carbohydrate and reducing sugar were compared to those from an untreated control.

(D) GROWTH OF SELECTED ANAEROBES ON PEAT HYDROLYSATES

(i) **Bacterial strains**

Clostridium acetobutylicum IFO 3854 was obtained from the Institute for Fermentation, Osaka, Japan.

C. acetobutylicum NRC 2484 (ATCC 10132) and C. butylicum NRC 33007 were obtained from the Division of Biological Sciences, National Research Council of Canada, Ottawa. C. butylicum NRRL B593 was obtained from the Northern Regional Research Laboratory, Preoria, Illinois. Clostridium thermohydrosulfuricum ATCC 33223 and Clostridium thermocellum ATCC 27405 were obtained from the American Type Culture Collection. Clostridium thermosaccharolyticum ATCC 7956 and Bacteroides succinogenes S85 were obtained from the departmental culture collection.

(ii) **Culture medium and growth conditions**

The culture media used are described in Tables 9A and 9B. The various media were inoculated with 10% (vol/vol) inocula and incubated without shaking at 36°C in the case of the C. acetobutylicum and C. butylicum cultures, and at 65°C in the case of C. thermohydrosulfuricum and C. thermosaccharolyticum. The cultures were adapted to grow on the peat media by three serial subcultures at 24 to 48 h intervals before performing experiments to analyse for fermentation products. For analysis of fermentation products, 1 mL samples were removed at the indicated times, centrifuged (16K x g, 5 min) to remove the cells and stored frozen.

(iii) **Product Analysis**

Fatty acids and solvents were determined by gas chromatography using a Hewlett Packard 5721A gas chromatograph equipped with a flame ionization detector. The stationary phase was Chromosorb 101 in a 2mm x 2m glass column. The injection port was heated at 200°C, the oven at 180°C and the detector at 250°C. Samples of culture fluid were acidified with sulfuric acid (0.1 M final concentration) and an internal standard of propionic acid (0.01M) was added to each sample prior to injection of 1 ul sample volume.

A typical separation of standard solutions is illustrated in Figure 3. Final concentrations of fermentation products were determined using a Hewlett Packard Model 3390 recording integrator. For determination of ethanol in C. thermohydrosulfuricum cultures, the separation and analysis conditions were as described above except that the oven temperature was 140°C.

(iv) **Substrate analysis**

For analysis of monosaccharides the samples (1 mL) were passed through Pasteur pipette columns composed of a mixed bed resin (Amberlite IR-120, hydrogen form and Rexyn 201, carbonate form) and made to 5 mL, filtered through 0.45u pore size membranes and 80 ul injected into the HPLC. For a description of the elution conditions, see II(B)(ix) above.

(v) **Preparation of mixed sugars solution**

To determine if the incomplete carbon utilization and low solvent yield of C. thermohydrosulfuricum noted earlier were due to the presence of inhibitors in the peat hydrolysate. A concentrated sugar solution containing the proportions of cellobiose, glucose, xylose, galactose, arabinose and mannose found in peat hydrolysates produced by the Stake process at a temperature of 200°C for 3 min, was used to supplement the GS medium described earlier (Table 10).

(vi) **Preparation of GS medium containing peat extract or mixed sugars solution as the source of carbohydrate**

The components required for 300 mL of GS medium were made to a volume of 250 mL, divided into 2 x 125 volumes sparged with N_2, boiled and autoclaved at 121°C for 17 min. Following cooling, 25 mL of a filter-sterilized solution containing peat hydrolysate (12.0% carbohydrate w/v) was added

to the other. Tubes of medium (4.5 mL) were dispensed aseptically under N_2.

(vii) **Growth of C. thermohydrosulfuricum in GS medium containing 2% peat extract carbohydrate or 0.67% mixed sugars solution**

C. thermohydrosulfuricum 33233 was serially transferred through appropriate medium containing either peat extract or the mixed sugar solution as the sole source of carbohydrate. Tubes were then innoculated in triplicate with 0.5 mL of a 24 h culture and incubated 4 days (96 h) at 60°C. Samples were removed before and after the fermentation and were analysed for soluble saccharide depletion by high pressure liquid chromotography and for product yield by gas chromotography.

RESULTS

(i) Analysis of a peat sample subjected to the wet carbonization process, and results from preliminary studies

Table 2 is a copy of the chemical analysis of the peat extract provided by Mr. McKim, of the Ontario Research Foundation. It is presumed to be representative of the extract we were to use as a nutrient source for fermentation studies. The total solids was 0.904% (wt/vol). No value was given for total carbohydrate, but the individual sugars and sugar acids (including glucuronate, galacturonate, rhamnose, and glucose) totalled 0.18% (wt/vol). No arabinose or pentoses were detected, and furfurals were less than 0.005% (wt/vol). The ammonium nitrogen concentration was 0.025% (wt/vol).

Table 3 presents one set of analyses performed in our laboratory on the peat extract provided. Our preliminary results complement those provided by Mr. McKim. An important feature is that the total carbohydrate is no greater than 0.24% (wt/vol).

For an economically sound fermentation process it is generally felt that at least 5% of fermentable carbohydrate is required to enable the cost effective recovery of the fermentation products. Because of the low carbohydrate in the extract we are of the opinion that use of the peat extract for solvent production would not be economically feasible.

(ii) Release of carbohydrate from peat by the Stake Process

Samples subjected to the steam explosion process were separated into solid and supernatant fractions as outlined in

the scheme illustrated in Fig. 1. The pH, dry weight, and total carbohydrate released during the process were determined and the results presented in a tabular form (Tables 4). The pH of the solubilized samples was generally about 1.35 pH units lower than untreated peat suspended in an equivalent volume of water (Table 4). The quantification suffered from incomplete sample recovery following processing. Total recovery of dry material ranged from 43 to 75%. The higher the temperature, and the longer the treatment, the lower the recovery. This was due to a combination of volatilization of material and difficulty of recovering all particulates from the cyclone collection device which trapped the samples on explosive release from the thermal processor. The ash content of the recovered materials (total soluble and insoluble) was low, ranging from 2.5 to 3.5%.

The amount of carbohydrate solubilized during processing increased as the temperature and time of treatment was increased to a maximum at 215°C and an exposure time of 1 min (Table 4). Longer exposure times at this temperature resulted in a net decrease in the solubilized carbohydrate concentration, presumably as a consequence of increased destruction of free sugars and volatilization of materials. The reducing sugar estimation, which measured the concentration of free monosaccharides, reached a maximum after treatments at 200°C for 2 to 3 min. Brief acidic hydrolysis of the solubilized carbohydrate samples did not increase the reducing sugar concentration further, but instead led to extensive destruction of sugar in samples steam treated at 215°C. Noteably, practically all of the material solubilized by the steam explosion treatment was carbohydrate in nature.

The comparative carbohydrate compositions of the peat extract and the residue are presented in Table 5. The residue was high in total carbohydrate and approximately half of this was cellulose. There was little glucosamine in the residue fraction indicating that invertebrate exoskeletons were not a significant component in the residue from this sample of peat.

(iii) **Sample pretreatment for HPLC analysis**

Samples were prepared for HPLC by passage through a mixed bed ion exchange resin. The commercially available mixed bed resin tested with standard sugar soutions was Amberlite MB-3. As shown in Table 6, there were reduced recoveries of cellobiose, xylose, and galactose. A number of mixed bed resins were then tested for binding of soluble carbohydrate from peat extracts (Table 7). As shown in Table 7, a mixed bed resin composed of Rexyn-201 (CO_3 form) and Amberlite IR-120 (H form) allowed the best recovery of reducing sugars. Analysis by HPLC of samples run through a mixed-bed Rexyn 201 (CO_3 form) and Amberlite IR-120 (H) form showed that the treatment did not result in a selective loss of any of the component sugars. These data show that practically all of the total carbohydrate is in the form of neutral sugars and uncharged polysaccharides.

After a number of samples, treated in the way described above, were run on the HPLC a high back pressure began to develop which was attributed to a binding of hydrophobic lignin and humic acid materials to the Aminex column resin. Acid pretreatment of samples gave rise to a precipitate which could be removed. This resulted in a reduction of the aromatic concentration in the samples (Fig. 5). The combination of precipitation of samples with 1 M sulfuric acid followed by neutralization and de-ashing with a

mixed-bed resin (Fig. 6) was found to be superior to either acid precipitation or de-ashing with a mixed-bed resin, or using acid precipitation followed by passage through an XAD 2 resin. The precipitation and de-ashing steps also resulted in a considerable reduction in the colour of the material.

From this study the best sample treatment evolved for HPLC analysis of sugars consisted of a two step process.

(1) Acidification of the sample to pH 2.0 with 1 M H_2SO_4. Precipitated lignin was removed by centrifugation and the supernatant fluid saved.

(2) Treatment of the supernatant fluid from (1) with a mixed bed ion-exchange resin consisting of equal parts of Rexyn-201 (carbonate form) and Amberlite IR-120 (hydrogen form) to remove cations and anions. The sample was then concentrated by vacuum evaporation, filtered through a Metricel GA-6 membrane of pore size 0.45 um (Gelman Sciences, Inc., Montreal, P.Q.) to remove all particulates prior to injection into the HPLC.

(iv) **Release of soluble saccharides by the Stake process**

Figure 3 illustrates the HPLC separation of sugars obtained from the peat extract prepared by heating peat at 200°C for 3 min. Peaks 1, 2, and 3, which did not correspond to known sugars, may represent neutral polysaccharides, perhaps hemicellulosics. The quantities of sugars detected in the peat extracts are shown in Table 8. It can be seen that the free sugars released increased with increased intensity of the treatment up to 3 min at 200°C. Beyond this, there was some apparent destruction of the sugars. However, the relative proportions of the determined sugars were similar to

those of samples processed at lower temperatures. As can be calculated from the data in Table 8, the treatment at 200°C for 3 min, released 6.0% of the dry weight (0.449/7.5 x 100) of the peat as free sugars consisting of cellobiose, glucose, xylose, galactose, arabinose and mannose. As shown in Table 4, the total solubilized carbohydrate of this extract was 18.1% of the total dry weight. Thus 33% of the total solubilized carbohydrate was released in the form of free sugars during the steam treatment at the optimum treatment condition. It is worth noting that the free sugars detected by HPLC constitute 55% of the total determined reducing sugar suggesting that the polysaccharide material released has a short chain length.

(v) **Peat extract as a substrate for growth and solvent production by clostridia**

In initial experiments a number of clostridia were tested for the ability to grow in the presence of peat extract since it was expected that inhibitors at high peat concentrations would prevent growth. All strains tested including Clostridium acetobutylicum strains 2484 and 3854, Clostridium butylicum strains B593 and 33007, and the thermophilic clostridia Clostridium thermosaccharolyticum 7956 and Clostridium thermohydrosulfuricum 33223, after acclimatization, grow in media with peat extract as the sole carbon source at concentrations of carbohydrate up to 2% (wt/vol). With the exception of C. thermohydrosulfuricum which has a requirement for tryptone and yeast extract, all strains tested were maintained on chemically defined media. During the growth of all bacteria in medium with peat extract there was a change in colour of the medium from a black molasses colour to a light tan suggesting that caramelized sugar was used by the organisms.

To determine the extent of utilization of carbohydrate in the peat extract by the clostridia, the strains that showed the best growth were subcultured in growth-limiting concentrations of the peat carbohydrate (Table 11) in the medium described in Table 9(a). The C. butylicum strains 33007 and B593 utilized approximately 20 to 23% of the total carbohydrate while strains 7956 and 33223 utilized between 30 and 50% of the total carbohydrate, respectively. A greater proportion of the reducing sugar was utilized.

To examine solvent production by the various strains including C. acetobutylicum strains 3854 and 2484, and C. butylicum strains B593 and 33007, peat extract was sterilized and added to the same sterile basal medium used previously to yield a carbohydrate concentration of 1.0% (wt/vol). Approximately 30 to 40% of the carbohydrate was utilized and was converted to acids and solvents with an efficiency of 30% (Fig. 7). This efficiency is typical of the acetone/butanol fermentation. Ethanol was not detected in any of the fermentations. This was due to a low product yield rather than an inability of the bacteria to produce the solvent. The low conversion of acetate and butyrate to solvents was expected since the final pH of the fermentation was greater than 5.0.

Clostridium thermohydrosulfuricum utilized about 50% of the carbohydrate during growth and produced ethanol to give a final concentration of 19.65 mM (=0.10%, wt/vol). Acetate was produced at a lower concentration than expected. No lactate was detected. Ethanol and acetate accounted for only 16% of the substrate utilized. Since not more than 15 to 20% of the substrate would be converted to cellular biomass, this leaves approximately 60 to 65% of the substrate utilized unaccounted for. (Please refer to the discussion for a consideration of these results).

(vi) **Carbohydrate utilization by C. thermohydrosulfuricum 33223 during growth on peat hydrolysate and mixed sugars solution**

C. thermohydrosulfuricum is reported to produce up to 1.9 moles of ethanol per mole of carbohydrate fermented (Ng et al., 1981). However, a much poorer yield was achieved in this study. To determine whether the poor ethanol yield was due to interfering compounds in the peat extract, C. thermohydrosulfuricum was grown on a carbohydrate mixture which simulated the carbohydrate complex found in peat (Table 13). In the simulated peat medium, all sugars except galactose were utilized very efficiently. Only glucose was utilized efficiently in the peat hydrolysate. The major monosaccharide in peat, galactose, was utilized very poorly.

(vii) **The use of peat residue as a substrate**

The use of the peat residue from the steam explosion process as a source of cellulose carbon for the growth of Clostridium thermocellum and Bacteroides succinogenes was tested using the culture medium of Scott and Dehority (1965) with 1.0% peat residue in the place of crystalline cellulose. The medium was inoculated with a 10% inoculum from cells grown in the same medium, except that Avicel crystalline cellulose was the source of carbon. No growth was detected with peat residue as the sole source of carbon. This would indicate that either there is an inhibitor in the residue preventing cell growth or there is insufficient cellulose in the residue to support growth of either strain.

Separate chemical determination of cellulose in the residue by extractive removal of associated polymers and determination of the cellulose using the phenol-sulfuric acid method revealed that 24% of the residue was cellulose thus indicating that there was enough cellulose to support growth of C. thermocellum and B. succinogenes.

DISCUSSION

For industrial fermentation a minimum of 5% of available carbohydrate must normally be present in the medium to ensure economical recovery of product (at a low concentration). The ideal carbohydrate concentration is usually considered to be in the range of 10 to 15% (wt/vol). This precludes the use of highly decomposed fuel grade peat or materials from wet carbonization processes as the carbohydrate concentrations are very low in these sources.

The best peat for use as fermentation substrates are the undegraded forms similar to horticultural grade peat. The data from this study has demonstrated that steam explosion (explosive decompression) releases up to 26% of the peat in a soluble form, the bulk of which (73%) is carbohydrate in nature. Approximately 33% of the total carbohydrate is present as free monosaccharides. Uronic acids were present in the solubilized material but no free glucosamine was detected. The residue remaining after the steam treatment still contained 53% by weight of total carbohydrate, of which a portion was cellulose.

The steam treatment necessary to give this degree of solubilization appeared to be less vigorous than that required to dissociate wood chips (Lora and Wayman, 1978) to release the hemicellulosic fraction.

The free sugars detected in the peat extract included galactose, glucose, xylose, mannose, arabinose and cellobiose in descending order of concentration. The relative abundance of sugars corresponds favourably with that reported for hydrochloric acid hydrolysates of peat reported by Bystraya and Rakovskii (1968; cited from Fuchsman, 1980), but differs

from the compositions of sulfuric acid hydrolysates of various peats reported by Morita and Montgomery (1980) and Morita and Levesque (1980). These workers found glucose to be the predominant sugar followed by galactose, mannose and xylose or arabinose. In our case, no acid hydrolysis was performed. Therefore, the higher concentrations of glucose normally associated with cellulose hydrolysis were not observed in the present study.

The complexity of the mixture of sugars, and in addition, polysaccharide materials released during the steam explosion process makes the selection of microorganisms for microbial fermentation difficult because few have the enzymatic diversity to utilize this range of carbohydrate substrates. For example, the mixture is unsuitable for a yeast fermentation, or a Zymomonas based fermentation, since these organisms utilize primarily only glucose as a substrate for the production of alcohol. However, many of the clostridia are well equipped for metabolizing complex substrates (Table 1). For this reason, Clostridium acetobutylicum, C. butylicum, C. thermosaccharolyticum and C. thermohydrosulfuricum were used in this study.

Water extracts from the steam explosion of wheat straw and aspen wood chips are reported to be very toxic, and restricted both the hydrolysis of the polysaccharide material, and the subsequent fermentation of the derived sugars (Mes-Hartree and Saddler, 1983). In contrast, Clostridium species grew well on peat extract as the source of carbohydrate in the culture medium. However, it was necessary to concentrate the extract by vacuum evaporation prior to analysis and fermentation, and it is possible that volatile inhibitors were removed at this stage.

Substrate utilization by the clostridia ranged from 30 to 50%, a rather low degree of carbohydrate utilization. Ideally, one would desire utilization of 70% or more of the substrate. With the great mixture of carbohydrates in a batch culture, catabolite repression of the utilization of other carbohydrates by glucose might be expected, although Ounine et al., (1983) claim that this is not a problem but they were using a different substrate mixture. However to avoid the possibility of catabolite repression, it is proposed that for scale-up experiments, when more substrate is available, a chemostat be used with a carbohydrate limitation thereby avoiding any potential of catabolite repression. This will also ensure synthesis of all inducible polymer hydrolyzing enzymes possessed by the clostridia. This approach has two advantages. Firstly, as just outlined, it will derepress a large number of hydrolytic enzymes. Second, it will continuously provide strong selective power for growth of mutants better able to utilize the limited substrate available. This was not attempted in the present study because an adequate amount of substrate was not available.

The yield of butanol, acetone and ethanol from the C. acetobutylicum and C. butylicum strains in this study was lower than that from a wood hydrolysate reported by Yu et al. (1984). Yu et al. (1984) used a sulfuric acid hydrolysate of a water washed cellulosic fraction of wood and the final processed sugar was of high purity. A low pH is a requirement for efficient solvent production (Bahl et al. 1982) and a low pH was not reached in the fermentations conducted in this study. The problem with pH control will be solved once adequate substrate is available to allow scale-up to fermentors with pH control. Although not reported in the literature (and contrary to the report by Fond et al. (1983)) it is possible to achieve high solvent

production from glucose by cultivation of clostridia with a carbon-limitation, when there is suitable pH control (unpublished data). Therefore, it is thought that there is still considerable flexibility to improve the conversion of peat extract to solvents. The peat residue should provide a cellulosic fraction similar to that used by Yu et al. (1984) and permit conversion to equal or better yields of solvent.

Clostridium thermohydrosulfuricum utilized the peat extract to the greatest extent of cultures tested. Nevertheless, the utilization of monosaccharides present in the peat extract was lower than that from a corresponding mixture of purified sugars. The reason for this is unknown. Fthanol production was produced in a lower yield than expected. C. thermohydrosulfuricum is reported to produce 1.5 moles of ethanol per mole of glucose (Ng et al. 1981) although values of 1.9 have been reported. The low ethanol production was not linked directly to an affect of inhibitors since ethanol production from an artifical mixture of sugars was also low. As with the C. butylicum species, chemostat culture may be valuable in increasing carbohydrate utilization and ethanol production.

A recent, interesting report (Lovitt et al., 1984) describes the successful isolation of solvent tolerant mutants of C. thermohydrosulfuricum by serial transferring through media containing successively higher concentrations of solvent. The mutant had enhanced ethanol producing ability. This may have important application in acclimatization of strains to high-substrate peat fermentations. In particular it lends optimism to the further development of this bacterium for the production of ethanol from peat sugars.

The insoluble residue remaining after the steam explosion of peat did not support the growth of either C. thermocellum or Bacteroides succinogenes. This was unexpected as the residue contained 24% cellulose, and the test organisms used are cellulolytic.

The peat residue becomes a black mush once it has been autoclaved in culture medium, and it would not be surprising if inhibitors were released during this treatment. The material contains, in addition to cellulose, waxes, lignin, and humic acids. It would be beneficial to remove these materials by organic and aqueous extractions before assessing the digestibility of the cellulose. It is very likely that the residual cellulose would be readily digested after such pretreatment.

What is the advantage of the Stake Process if it does not result in complete hydrolysis of all polymers to simple sugar?

The Stake process releases from peat approximately 25% of its dry weight as soluble material. Of this extracted material, 75% is non-cellulosic carbohydrate. The separation of cellulose from the low molecular weight carbohydrates is a potential advantage in subsequent fermentations involving these fractions as substrates: the presence of the sugars would probably result in the inefficient use of cellulose by the fermenting organisms. Fortunately, the peat extracts are free from inhibitors of microbial growth, thus obviating the need for expensive procedures for their removal.

Optimisation of the fermentation of peat extracts will require that additional, relatively large, quantities of this material (Kg quantities) be available.

Clostridium thermohydrosulfuricum very efficiently utilized the solubilized peat extract but did not produce ethanol efficiently. This requires further research. Fortunately new strains are becoming available which will resolve this problem. Furthermore, other types of fermentations may need to be undertaken.

The cellulose in the peat residue was not available as a substrate for cellulolytic bacteria. If the residue is first extracted to remove valuable waxes and lignin, it is very likely that the residual cellulose will be readily fermentable.

To quantitatively collect the steam exploded materials from the small scale cyclone collector at Stake Technology Ltd., a considerable amount of water was needed. In the pilot and production scale processes, the only water used is in the form of steam and there is therefore little resultant dilution of the exploded materials. Thus, the soluble carbohydrate concentration can be maintained at high levels under commercial production conditions.

In summary, it is considered that the Stake process will allow the systematic fractionation of peat into the following component parts:

1) Soluble carbohydrates fermentation substrate
2) Waxes - by-product
3) Lignin - by-product
4) Humic acids - by product
5) Cellulose - fermentation substrate

We have shown that some bacteria can utilize the soluble carbohydrate fractions, but further research is necessary to develop efficient fermentations.

REFERENCES

Andersch, W., H. Bahl, and G. Gottschalk. 1982. Acetone-butanol production by Clostridium acetobutylicum in an ammonium-limited chemostat at low pH values. Biotechnol. Lett. 4 : 29-32.

Ashwell, G. 1957. Colorimetric analysis of sugars. Methods Enzymol. 3 :73- 105.

Bahl, H., W. Andersch, and G. Gottschalk. 1982. Continuous production of acetone and butanol by Clostridium acetobutylicum in a two-stage phosphate-limited chemostat. Eur. J. Appl. Microbiol. Biotechnol. 15 : 201-205.

Davidson, E.A. 1966. Analysis of sugars found in mycopolysaccharides Methods Enzymol. 8 : 55.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for the determination of sugars and related substances. Anal. Chem. 28 : 350-356.

Fuchsman, C.H. 1980. Peat: Industrial Chemistry and Technology. Academic Press. New York.

Garcia-Martinez, D.V., A. Shinmyo, A. Madia, and A.L. Demain. 1980. Studies on cellulase production by Clostridium thermocellum. Eur. J. Appl. Microbiol. Biotechnol. 9 :189-197.

Gould, J.M. 1984. Alkaline peroxide delignification of agricultural residues to enhance enzymatic saccharification. Biotech. Bioeng. 24 : 46-52.

Grethlein, Hans E. 1978. Chemical breakdown of cellulosic materials. J. Appl. Chem. Biotechnol. 20 : 296-308.

LeDuy, A. 1979. SCP from peat hydrolysates. Proc. Biochem. 14 : 5-7.

Lora, J.H., and M. Wayman. 1978. Delignification of hardwoods by autohydrolysis and extraction. Tappi 61 : 47-50

Lovitt, R.W., R. Longin, and J.G. Zeikus. 1984. Ethanol production by thermophilic bacteria: physiological comparison of solvent effects on parent and alcohol-tolerant strains of Clostridium thermohydrosulfuricum. Appl. Environ. Microbiol. 48 : 171-177.

Maehly, A.C. and B. Chance. 1954. The assay of catalases and peroxidases. In Methods of Biochemical Analysis Vol 1: 357-424.

Mes-Hartree, M. and J.N. Saddler. 1983. The nature of inhibitory materials present in pretreated lignocellulosic substrates which inhibit the enzymatic hydrolysis of cellulose. Biotechnol. Lett. 5: 531-536.

Morita, H., and W.G. Montgomery. 1980. Monosaccharide composition of selected Canadian peats. Can. J. Soil Sci. 60: 1-7.

Morita, H., and M. Levesque. 1980. Monosaccharide composition of peat fractions based on particle size. Can. J. Soil Sci. 60: 285-289.

Murray, W.D., and M. Asther. 1984. Ethanol fermentation of exose and pentose wood sugars produced by hydrogen-fluoride solvalysis of aspen chips. Biotechnol. Lett. 6: 323-326.

Ng, T.K., A. Ben-Bassat, and J.G. Zeikus. 1981. Ethanol production by thermophilic bacteria: Fermentation of cellulosic substrates by co- cultures of Clostridium thermocellum and Clostridium thermohydrosulfuricum. Appl. Environ. Microbiol. 41: 1337-1343.

Ounine, K., H. Petitdemage, G. Ravel and R. Gay. 1983. Acetone-butanol production from pentoses by Clostridium acetobutylicum. Biotechnol. Lett. 5: 605-610.

Rondle, C.J.M. and W.T.J. Morgan. 1955. The determination of glucosamine and galactosamine. Biochem. J. 61: 586.

Scott, H.W., and B.A. Dehority. 1965. Vitamin requirements of several cellulolytic rumen bacteria. J. Bacteriol. 89: 1169-1175.

Spivey, M.J. 1978. The acetone/butanol/ethanol fermentation. Process Biochem. Nov. 13: 2.

Updegraff, D.M. 1969. Semimicro determination of cellulose in biological materials. Anal. Biochem. 32: 420-424.

Weatherburn, M.W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem. 39: 971-974.

Yu, E.K.C., L. Deschatelets, and J.N. Saddler. 1984. The bioconversion of wood hydrolyzates to butanol and butanediol. Biotechnol. Lett. 6: 327-332.

Zeikus, J.G., A. Ben-Bassat, T.K. Ng. and R.J. Lamed. 1981. Thermophilic ethanol fermentations, p.441-462. In A. Hollander (ed.), Trends in the biology of fermentation for fuels and chemicals. Plenum Publishing Corp., New York.

Zeikus, J. G. 1979. Thermophilic bacteria: ecology, physiology and technology. Enzyme Microbial Technol. 1: 243-252.

APPENDIX I

Alkaline delignification of peat

One of the objectives of this period was to examine delignification of peat by means of a novel alkaline-peroxide treatment. Gould (1984) reported that up to 50% of the lignin found in agricultural wastes could be selectively removed by means of a simple peroxide treatment conducted at ambient temperatures (25°C) - a method that does not suffer from the disadvantages inherent in pretreatment processes which require a high energy input. Peroxide pretreatment has been found to increase the "fermentability" of the resultant residue from 49.8 to 100% in the case of waste corn stalks with similar improvements reported for other types of crop residues (Gould 1984).

We have applied a similar treatment to peat (Figure 2) with the objective of obtaining a less refractile substrate for subsequent solvent fermentations. The mechanism of action of the peroxide is simple. Hydrogen peroxide, under alkaline conditions, dissociates to form a highly reactive hydroxyl radical (HO·) which reacts with lignin to form soluble oxidation products. After treatment, the supernatant fraction, containing hemicellulose and low molecular weight phenolics from lignin oxidation, can be used to treat additional subtstrate. After several cyclings, a concentrated extract of hemicellulose is obtained which, after acid hydrolysis to constituent monosaccharides, could conceivably be employed in pentose fermentations. The insoluble delignified residue, consisting of cellulose and some residual lignin, can be used to support growth of cellulolytic bacteria.

Hydrogen peroxide was measured by titration with potassium permanganate (Maehly and Chance, 1954). Samples (50 ml) containing 0.68 g of hydrogen peroxide were titrated to a colourless endpoint with 0.01 N potassium permanganate in 0.01 M sodium phosphate buffer (pH=7.0). The results (titrant consumed) were related to a standard curve which had been prepared using a stock solution of 30% hydrogen peroxide in water.

Hydrogen peroxide estimations made before and after each 24th extraction cycle indicated that virtually all of the oxidant had been consumed during the delignification.

Conclusions:

1. The physical appearance of the delignified material was markedly changed from a fibrous to an amorphous state.

2. A large amount of hydrogen peroxide is used in this process.

3. The treated material was not tested to determine whether it was fermentable.

APPFNDIX II

TABLES

TABLE 1 : Growth characteristics of *Clostridium* species used for solvent production[a]

Characteristics	*C. butylicum* NRRL B593	*C. thermocellum* ATCC 27405	*C. thermosaccharolyticum* ATCC 7956	*C. thermohydrosulfuricum* ATCC 33223
Temp. range for growth:				
Min		40°C	-	40°C
Opt	35°C	62°C	58°C	65°C
Max		70°C	65°C	75°C
Substrates:				
(a) Supporting growth	Cellobiose, sucrose, lactose, glucose galactose, mannose, fructose, xylose, arabinose, ribose, rhamnose, glucuronic acid, galacturonic acid, glucosamine starch, xylan, inulin	Cellulose cellodextrins cellobiose glucose	Cellobiose, lactose, maltose, glucose, fructose, mannose, galactose, xylose arabinose, starch	Cellobiose, sucrose, maltose, raffinose, glucose, galactose, mannose, fructose, xylose, ribose, pyruvate, starch, pectin
(b) Not Supporting growth	Cellulose	Xylose, mannose, sucrose, starch	Cellulose	Cellodextrins, cellulose, xylan, mannan
Fermentation Products	Butanol, acetone, ethanol, (acetoin), H_2, CO_2, acetic acid, butyric acid	Ethanol, acetate lactate, CO_2, H_2	Ethanol, acetate, lactate, butyrate, CO_2, H_2	Ethanol, acetate, lactate, CO_2, H_2

[a]Zeikus et al. 1981.

TABLE 2: Chemical analysis of a dewatering extract/pressate from fuel grade peat. Data provided by Mr. M. McKim of the Ontario Research Foundation (Feb. 20, 1984).

PEAT ANALYTICAL RESULTS
WET OXIDATION TRIALS

RUN No. SAMPLE DATE SAMPLED DATE ANALYSED WET METHODS:	(UNITS)	MINIMUM COMBINED	AVERAGE COMBINED	MAXIMUM COMBINED	MINIMUM CONDENSATE	AVERAGE CONDENSATE	MAXIMUM CONDENSATE
TOTAL SOLIDS	mg/L	7670	9040	9920	32	391.66667	1320
TOTAL VOLATILE SOLIDS	%	82	84.85	88.4	0	69.4	91
SUSPENDED SOLIDS	mg/L	64.5	276.75	550	24	283	976
VOLATILE SUSPENDED SOLIDS	mg/L	52	236.83333	470	24	278.33333	952
CHEMICAL OXYGEN DEMAND	mg/L	11900	12683.333	13600	4800	7813.3333	12900
TOTAL ORGANIC CARBON	mg/L	4050	4808.3333	5300	1700	2216.6667	2850
pH		3.07	3.3133333	3.67	2.68	2.84	3.1
ACIDITY, pH3. 7	mg CaC03/L	50	583.33333	800	350	461.33333	650
, pH8. 3	mg CaC03/L	3370	4220	5760	850	1560	2100
TOTAL VOLATILE ACIDS	mg AcH/L	2210	2658.3333	3180	875	1627.5	2260
COLOUR	Hazen	4500	6958.3333	10000	40	78.333333	110
PHENOLICS	mg/L	1.7	10.606667	23	1.35	74.758333	350
AMMONIA	mg N/L	180	254.83333	307	7	24.333333	55
TKN	mg N/L	400	482	547	8.4	34.333333	65
SULPHATE	mg/L	174	302.16667	470	5	6.8333333	10
SULPHILE	mg/L	0	0	0	0	0	0
TOTAL PHOSPHOROUS	mg/L	10	19.041667	25	0	.00666667	.04
FULVIC ACIDS	mg C/L	3300	4150	4700	870	1578.3333	2100
HUMIC ACIDS	mg C/L	60	461.66667	1200	180	470	780
*OIL & GREASE	mg/L		24			960	
BIOLOGICAL METHOD							
MICROTOX, 5 min EC50	%	.23	.36	.41	.16	.4	.54
MICROTOX, 15 min EC50	%	.25	.405	.52	.15	.34	.45
*HPLC							
GLUCRONATE	mg/L		288			N.D.	
ACETATE	mg/L		1480			1160	
GALACTARONATE	mg/L		735			N.D.	
ARABINOSE	mg/L		N.D.			N.D.	
RHAMNOSE	mg/L		542			N.D.	
PENTOSES (XYL, MAN, GAL)	mg/L		N.D.			288	
GLUCOSE	mg/L		206			62	
FURFURAL	mg/L		6.7			27.2	
HYDROXY METHYL FUFURAL	mg/L		38.5			38.1	

* not averages

TABLE 3: Analysis of a dewatering extract/pressate from peat. The extract was provided by Mr. J. Sheppard.

Component	Percent(wt/vol)
Total Solids[1]	0.82
Total Carbohydrate[2]	0.24
Ammonium Nitrogen[3]	0.007

[1]Determined by freeze drying samples.
[2]Phenol-sulfuric acid method of Dubois et al. 1956. Anal. Chem. 28:350-356.
[3]Phenol-hypochlorite method of Weatherburn. 1967. Anal.Chem. 39:971-974.

TABLE 4 - Release of dry weight, soluble carbohydrate and reducing sugar from steam-exploded samples[1]

Treatment °C	min	pH	Dry weight solubilized (g)	Carbohydrate[2] (g)	Reducing sugar before hydrolysis (g)	Reducing sugar after hydrolysis[3] (g)
170	0.5	3.52	0.213 (2.8)	0.095 (1.3)	0.021 (0.3)	0.045 (0.6)
170	1.0	3.50	0.208 (2.8)	0.132 (1.8)	0.025 (0.3)	0.065 (0.9)
170	2.0	3.57	0.413 (5.5)	0.245 (3.2)	0.086 (1.1)	0.079 (1.5)
170	3.0	3.52	0.530 (7.0)	0.364 (4.9)	0.121 (1.6)	0.145 (1.9)
170	5.0	3.34	1.160 (15.5)	0.878 (11.7)	0.332 (4.4)	0.287 (3.8)
200	0.5	3.44	1.038 (13.8)	0.687 (9.2)	0.291 (3.8)	0.551 (7.3)
200	1.0	3.32	1.733 (23.1)	1.300 (17.3)	0.530 (7.1)	0.699 (9.3)
200	2.0	3.37	1.783 (23.8)	1.360 (18.1)	0.724 (9.7)	0.781 (10.4)
200	3.0	3.39	1.790 (23.9)	1.360 (18.1)	0.822 (10.9)	0.832 (11.1)
200	5.0	3.39	1.933 (25.8)	1.41 (18.8)	0.709 (9.4)	0.822 (10.9)
215	0.5	3.39	1.23 (16.4)	0.927 (12.4)	0.378 (5.1)	0.542 (7.2)
215	1.0	3.42	2.09 (27.8)	1.53 (20.4)	0.609 (8.1)	0.764 (10.1)
215	2.0	3.40	1.77 (23.7)	1.30 (17.3)	0.704 (9.3)	0.523 (6.9)
215	3.0	3.40	1.72 (22.9)	1.20 (16.0)	0.784 (10.5)	0.376 (5.0)
215	5.0	3.42	1.51 (20.1)	1.15 (15.3)	0.714 (9.5)	0.382 (5.0)
Peat control[4]		4.85	0.045 (0.6)	0.030 (0.4)	0.006 (0.05)	

[1] - all values represent quantities released form a single 15.0 g sample (50% wt/vol, moisture) by steam explosion at the time and temperatures indicated. Figures in parenthesis represent the percentage of dry weight solubilized.

[2] - carbohydrate was determined as previously described. All values represent material recovered. Per cent solubilization was calculated by dividing the amount of carbohydrate released per 7.5 g dry wt sample of peat.

[3] - after 2.0 h hydrolysis of the sample in 2.0 N HCl at 100° C. Values reported have not been corrected for carbohydrate degradation during acid hydrolysis.

[4] - peat control was processed as treated samples and identical parameters measured.

TABLE 5: Composition of peat extract and peat residue obtained after treatment of peat at 200°C for 3 min followed by explosive decompression (see Fig. 1 for further details).

Treatment	Component[1]	Extract mg g^{-1}	Residue mg g^{-1}
None	Carbohydrate soluble	757.6	48.4
	insoluble	n.d.[2]	530.5
	Reducing sugar soluble	365.7	n.d.
	insoluble	n.d.	123.0
140°C, 60 min 0.6% (w/v) H_2SO_4	Carbohydrate soluble	450.0	112.5
	insoluble	94.3	280.8
	Reducing sugar soluble	365.1	77.1
	insoluble	76.6	126.5
121°C 120 min 1.6% (w/v) H_2SO_4	Carbohydrate soluble	662.2	
"	Reducing Sugar soluble	586.2	
"	Glucosamine		1.88
"	Glucosamine	1.6	
None	Glucosamine	n.d.	
None	Glucuronic Acid	83.4	
None	Ammonia	0.13	
	Cellulose		240.0

[1]- after acid hydrolysis samples were centrifuged (16K xg, 5.0 min) prior to carbohydrate and reducing sugar analysis.
[2]- not detected

TABLE 6: Recover of standard sugar solutions eluted from Amberlite MB3 columns. Samples of 20 1 of 5.0% (wt/vol) standard sugar solutions were applied to the top of the resin bed and were eluted to a volume of 10 mL with distilled water.

Sugar	Added mg	Recovered mg	% Recovered
Cellobiose	1000	761	76.1
Glucose	1000	864	86.4
Xylose	1000	779	77.9
Galactose	1000	783	78.3
Arabinose	1000	909	90.9
Mannose	1000	867	86.7

TABLE 7: Recovery of soluble carbohydrate and reducing sugar from peat extract samples passed through mixed-bed ion-exchange resins[1]. Experimental conditions are described in Materials and Methods.

Resin	Carbohydrate[2]	Reducing Sugar[3]	pH
Control[4]	12.1 (100)	6.8 (100)	6.5
Rexyn 201 (CO_3 form) -Amberlite IR-120 (H form)	11.3 (93.5)	5.7 (84.3)	3.2
Rexyn 201 (OH form) -Amberlite IR-120 (H form)	7.8 (64.2)	5.1 (75.6)	3.7
Amberlite MB3	5.8 (47.7)	4.4 (64.4)	4.3

1- Results are expressed as % (wt/vol) of concentrated extract.
2- Measured by the phenol-sulfuric acid method
3- Measured by the arsenomolybdate assay.
4- Untreated peat extract diluted to the same volume as samples eluted from mixed bed resins.

TABLE 8 : Free sugars in the peat extract determined by high pressure liquid chromatography.

Treatment °C	min	Grams of free sugar per 7.5 gm (dry weight) sample						
		Cellobiose	Glucose	Xylose	Galactose	Arabinose	Mannose	Total
170	0.5	0.002 (20.0)[1]	n.d.[2]	n.d.	n.d.	n.d.	n.d.	0.010
170	1.0	0.002 (20.0)	n.d.	n.d.	n.d.	n.d.	n.d.	0.010
170	2.0	0.006 (8.2)	0.008 (11.3)	0.005 (7.3)	0.033 (48.5)	0.011 (16.2)	n.d.	0.068
170	3.0	0.006 (8.0)	0.008 (10.5)	0.012 (15.5)	0.038 (49.3)	0.013 (16.8)	n.d.	0.077
170	5.0	0.016 (8.2)	0.023 (11.9)	0.033 (17.1)	0.094 (48.7)	0.027 (13.9)	n.d.	0.193
200	0.5	0.017 (9.5)	0.023 (12.9)	0.034 (19.1)	0.081 (45.5)	0.023 (12.9)	n.d.	0.178
200	1.0	0.023 (8.1)	0.043 (15.2)	0.058 (20.5)	0.129 (45.5)	0.014 (4.9)	0.016 (5.6)	0.283
200	2.0	0.027 (7.2)	0.063 (16.9)	0.063 (16.9)	0.154 (41.5)	0.025 (6.7)	0.039 (10.5)	0.371
200	3.0	0.031 (6.9)	0.074 (16.4)	0.071 (16.1)	0.187 (41.6)	0.036 (8.0)	0.050 (11.1)	0.449
200	5.0	0.029 (8.1)	0.052 (14.6)	0.065 (18.3)	0.140 (39.5)	0.030 (8.4)	0.038 (10.7)	0.354
215	0.5	0.018 (10.4)	0.035 (20.2)	0.023 (13.2)	0.073 (42.1)	0.008 (4.6)	0.016 (9.2)	0.173
215	1.0	0.028 (9.6)	0.063 (21.7)	0.042 (14.5)	0.112 (38.8)	0.016 (5.5)	0.028 (9.6)	0.289
215	2.0	0.028 (7.4)	0.071 (18.9)	0.050 (13.3)	0.119 (31.7)	0.032 (8.6)	0.061 (16.3)	0.375
215	3.0	0.028 (9.1)	0.071 (22.8)	0.043 (14.0)	0.121 (39.5)	0.013 (4.2)	0.031 (10.6)	0.306
215	5.0	0.022 (15.7)	0.035 (25.1)	0.014 (10.0)	0.055 (39.3)	0.006 (4.2)	0.008 (5.7)	0.139
Peat Control		n.d.	n.d.	n.d.	0.003 (100)	n.d.	n.d.	0.003

[1] - figures in parentheses denote the percentage of total free sugars that the saccharide constitutes.

[2] - not detected. Peaks having a height of less than 2.0 mm not recorded since quanitities of these minor components could not be properly assessed.

Table 9(a):

Culture medium for C. acetobutylicum and C. butylicum strains. (Andersch et al. 1982)

KH_2PO_4	1.0g
$K_2HPO_4.3H_2O$	1.0g
$Mg\ SO_4,7H_2O$	0.1g
NaCl	0.01g
$Na_2MoO_4.2H_2O$	0.01g
$CaCl_2.6H_2O$	0.01g
$Mn\ SO_4.H_2O$	0.015g
$FeSO_4.7H_2O$	0.015g
Biotin	0.1mg
Thiamine-HCl	2.0mg
p-Aminobenzoic acid	2.0mg
$(NH_4)_2\ SO_4$	2.0g
Glucose other CHO or Peat	- VARIED
Resazurin	1.0mg

pH = 6.5
volume to 1 litre with water

TABLE 9(b):

Culture medium for C. thermohydrosulfuricum (GS medium of Garcia-Martinez et al. (1980) modified by the addition of trytone and yeast extract).

KH_2PO_4	0.5g
$K_2H\ PO_4.3H_2O$	1.0g
Urea	2.0g
$MgCl_2.6H_2O$	0.5g
$CaCl_2.2H_2O$	0.05g
$FeSO_4.7H_2O$	1.25mg
Morpholinopropane-sulfonic acid	10.0g
Tryptone	10.0g
Yeast extract	3.0g
Carbohydrate	VARIED

pH = 7.2
volume to 1 litre with water

TABLE 10 : Composition of stock mixed sugar standard (10% carbohydrate)

	Weight (g)
Cellobiose	0.69
Glucose	1.64
Xylose	1.61
Galactose	4.16
Mannose	0.80
Arabinose	1.10
	10.0 g to 100 mL with distilled water

TABLE 11: Depletion of carbohydrate and reducing sugar by Clostridium butylicum 33007 and B593, Clostridium thermosaccharolyticum 7956 and Clostridium thermohydrosulfuricum 33223 during growth on peat extract.

Strain	Carbohydrate (%)	Time (h)	Total Carbohydrate (mg ml^{-1})	% Change	Total Reducing Sugar (mg ml^{-1})	% Change
33007	0.3	0	3.0		0.826	
		72	2.3	23	0.426	48.4
		120	2.3	23	0.401	51.1
33007	0.6	0	6.0		1.741	
		72	4.8	21	1.161	32.2
		120	4.8	21	0.989	12.3
B593	0.3	0	3.1		0.847	
		72	2.5	19	0.618	27.0
		120	2.4	20	0.516	39.1
B593	0.6	0	6.0		1.696	
		72	4.9	18	1.247	26.5
		120	4.8	20	1.089	36.0
7956	0.3	0	3.3		0.868	
		72	2.2	33	0.485	44.0
		120	2.2	33	0.399	54.0
33223	1.5	0	15.0		5.893	
		72	9.1	39	2.946	50.0
		120	7.8	48	2.660	55.0

TABLE 12 : Utilization of carbohydrate and the production of ethanol and acetic acid by Clostridium thermohydrosulfuricum 33223 during growth on 1.5% peat extract carbohydrate.

Carbohydrate (%)	Time (h)	Total Carbohydrate (mg ml^{-1})	% change	Total Reducing sugar (mg ml^{-1})	% change	Ethanol mM	Acetic acid mM
1.5	0	15.0		5.893		1.96	1.48
	72	9.1	39	2.946	50.0	19.65	6.06
	120	7.8	48	2.660	55.0	17.91	6.63

TABLE 13 : Utilization of constituent soluble saccharides of peat hydrolysates by Clostridium thermohydrosulfuricum 33223

Source of Carbohydrate	Type of Saccharide	Time of Incubation (h)	Concentration mM	Standard Error	% Depletion
Mixed Sugars	cellobiose	0	1.234	0.48	
		96	0.16	0.01	87
	glucose	0	3.75	0.06	
		96	0	n.d.	100
	xylose	0	4.68	0.18	
		96	0.56	0.44	78
	galactose	0	13.65	0.47	
		96	5.16	0.46	62
	arabinose + mannose [1]	0	3.21	0.03	
		96	0.85	0.69	73
Peeat Hydrolysate	cellobiose	0	1.40	0.06	
		96	0.61	0.26	56
	glucose	0	4.01	0.39	
		96	0.22	0.18	90
	xylose	0	3.77	0.33	
		96	1.64	0.29	56
	galactose	0	9.51	0.47	
		96	7.26	1.44	23
	arabinose + mannose	0	5.48	0.17	
		96	3.02	0.45	45

[1] Results expressed as arabinose equivalents since these two sugars could not be adequately resolved by HPLC.

TABLE 14 : Production of ethanol and acetic acid by Clostridium thermohydrosulfuricum 33223 during growth on peat hydrolysate and mixed sugars media.

Type of carbohydrate in medium	Time of Incubation	Ethanol mM	Acetic Acid mM
Mixed Sugars	0	1.29 (0.08)[1]	0.36 (0.08)
	96	22.48 (2.60)	8.52 (0.47)
	difference	21.19	8.16
Peat Hydrolysate	0	1.69(0.27)	1.73 (0.23)
	96	16.81 (2.9)	10.98 (0.94)
	difference	15.12	9.25

[1] figures in parentheses denote the standard error of the mean.

APPENDIX III

FIGURES

FIGURE 1 : Processing of 15g peat samples treated at 170, 200 and 215°C for 0.5, 1.0, 2.0, 3.0 and 5.0 min.

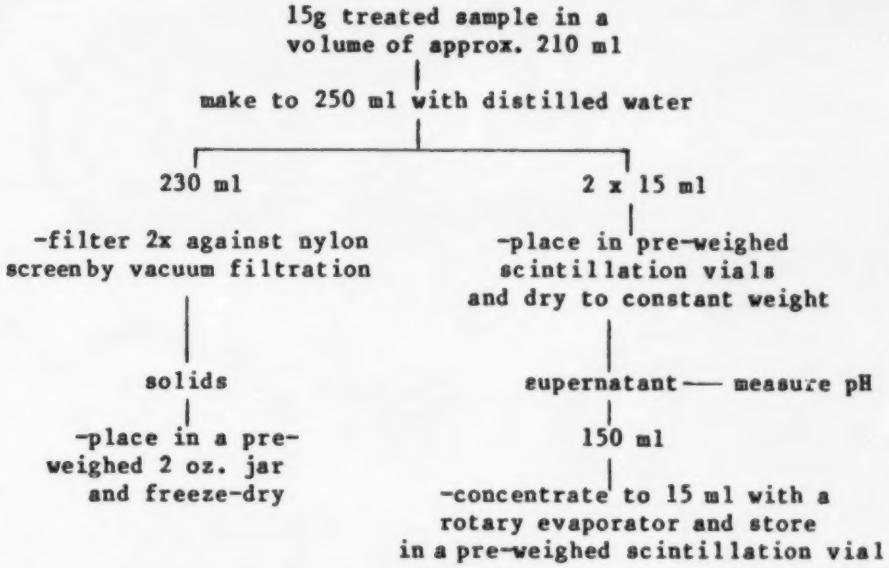
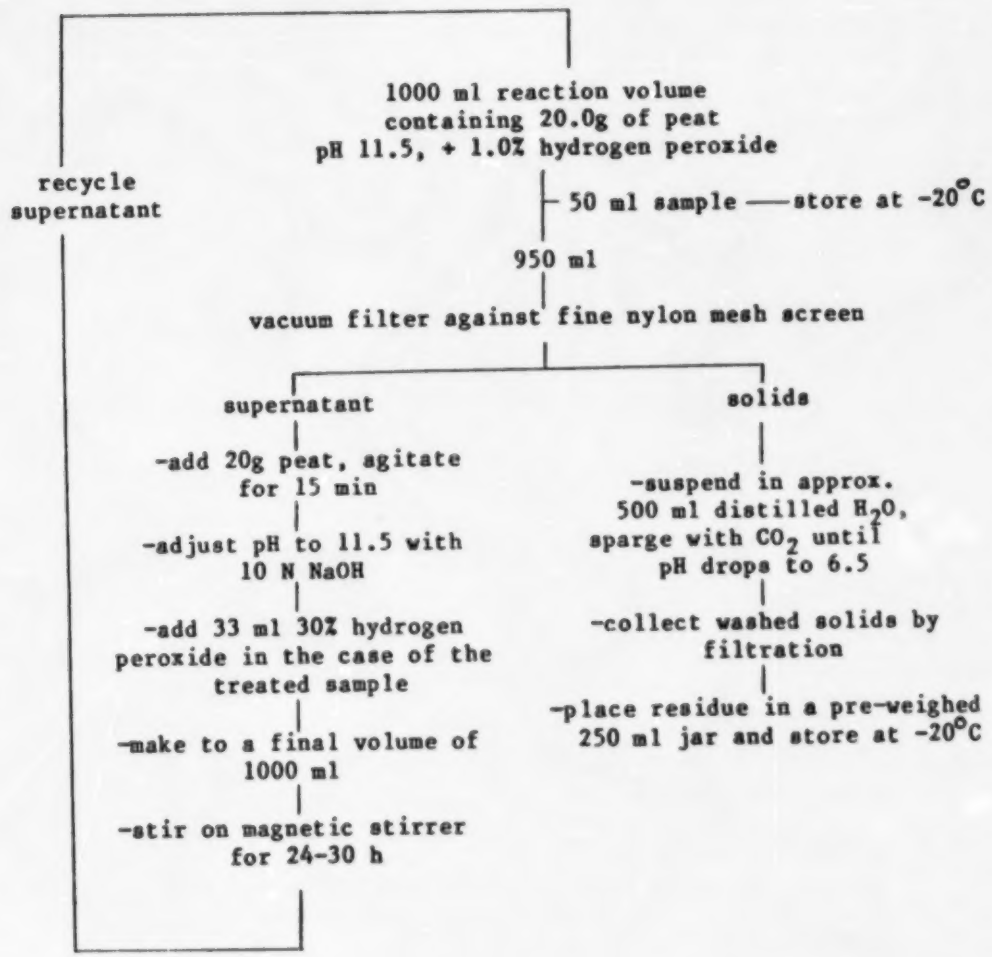

15g treated sample in a volume of approx. 210 ml
make to 250 ml with distilled water
230 ml
2 x 15 ml
-filter 2x against nylon screenby vacuum filtration
-place in pre-weighed scintillation vials and dry to constant weight
solids
supernatant — measure pH
-place in a pre-weighed 2 oz. jar and freeze-dry
150 ml
-concentrate to 15 ml with a rotary evaporator and store in a pre-weighed scintillation vial

FIGURE 2 : Flow chart of the methodology employed in the treatment of peat with 1% hydrogen peroxide under alkaline conditions.


1000 ml reaction volume
containing 20.0g of peat
pH 11.5, + 1.0% hydrogen peroxide
recycle supernatant
50 ml sample —— store at -20°C
950 ml
vacuum filter against fine nylon mesh screen
supernatant
solids
-add 20g peat, agitate for 15 min
-adjust pH to 11.5 with 10 N NaOH
-add 33 ml 30% hydrogen peroxide in the case of the treated sample
-make to a final volume of 1000 ml
-stir on magnetic stirrer for 24-30 h
-suspend in approx. 500 ml distilled H_2O, sparge with CO_2 until pH drops to 6.5
-collect washed solids by filtration
-place residue in a pre-weighed 250 ml jar and store at -20°C

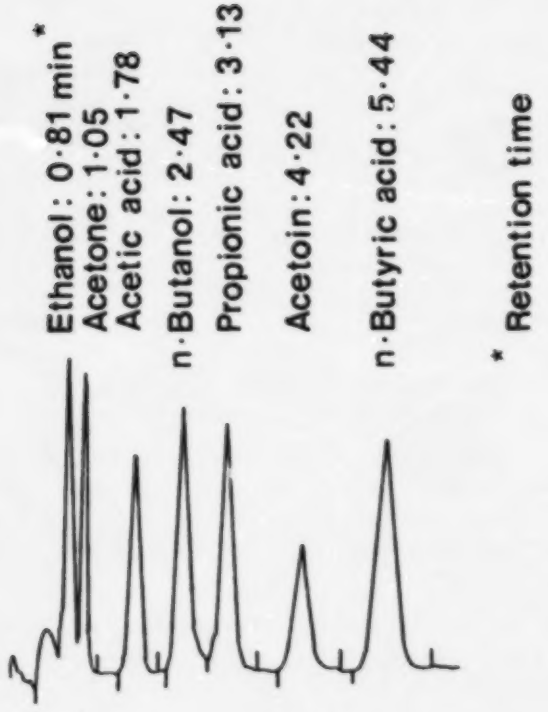


FIGURE 3 : A typical separation of fatty acid and solvent standards by gas chromatography with a Chromosorb 101 stationary phase. The numbers at the peaks represent the retention times in minutes.

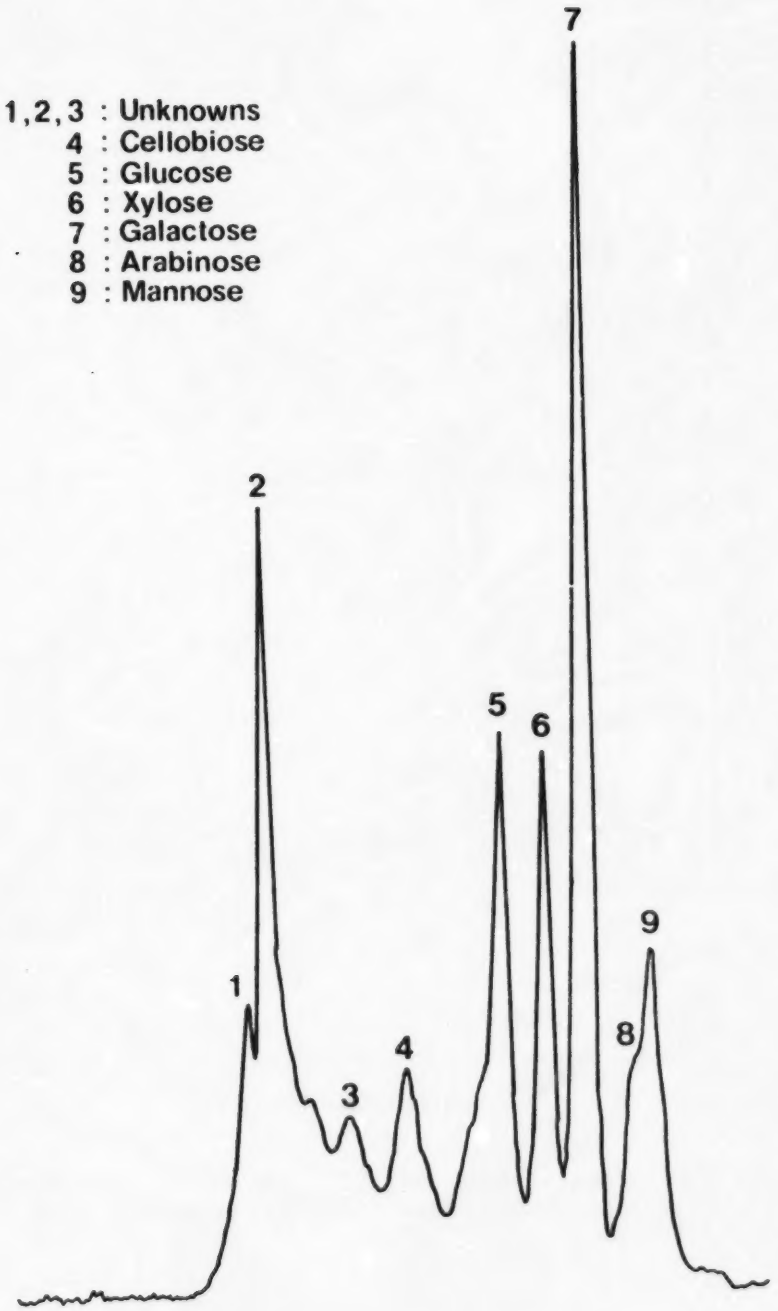


FIGURE 4 : A chromatogram illustrating the separation of sugars present in the soluble fraction after steam explosion of peat (heated for 3.0 min. at 200°C) by HPLC on a Biorad HPX-87P carbohydrate analysis column (temperature = 85°C). Peaks : unknowns - 1, 2 & 3; cellobiose, 4 ; glucose, 5; xylose, 6; galactose, 7; arabinose, 8 and mannose 9. A sample of 80 ul was applied at a flow rate of 0.6 mL min^{-1}. The attenuation was 8x.

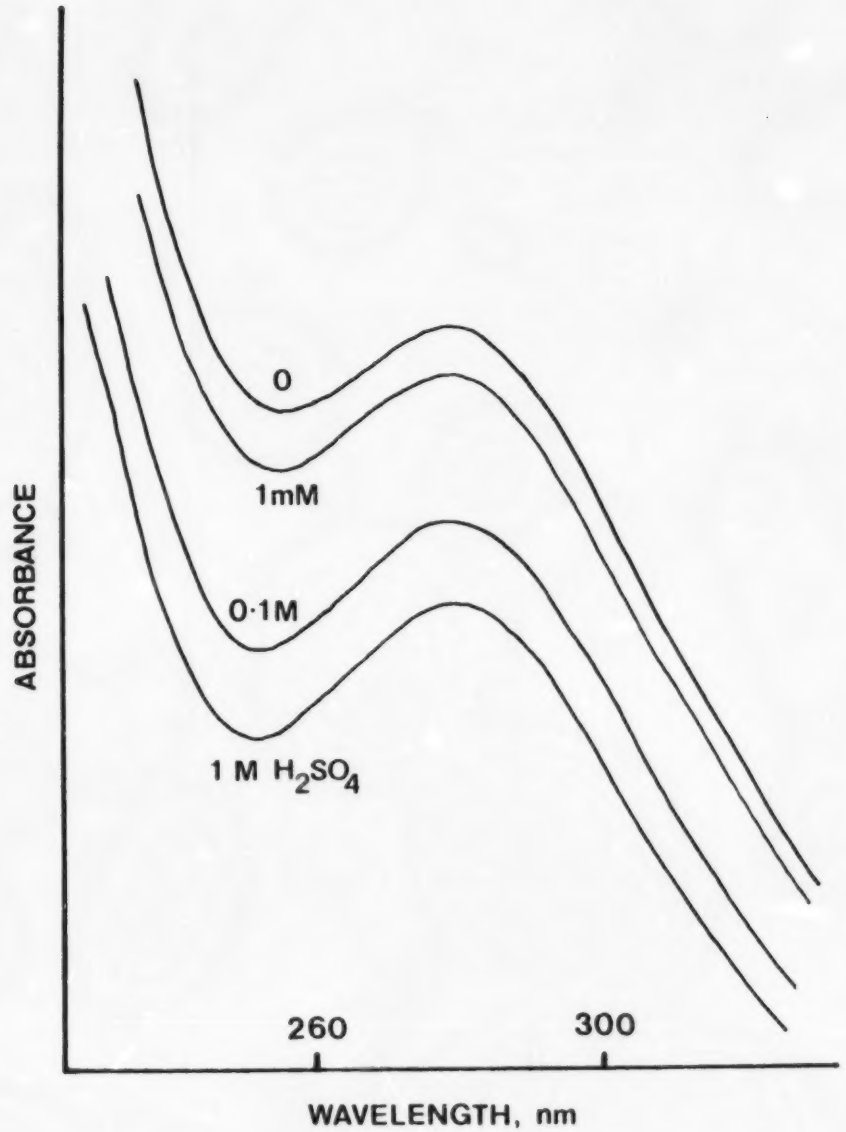


FIGURE 5 : The effect of acid concentration on the absorbance profile of peat hydrolysate dilutions. (Final carbohydrate concentration = 0.06%)

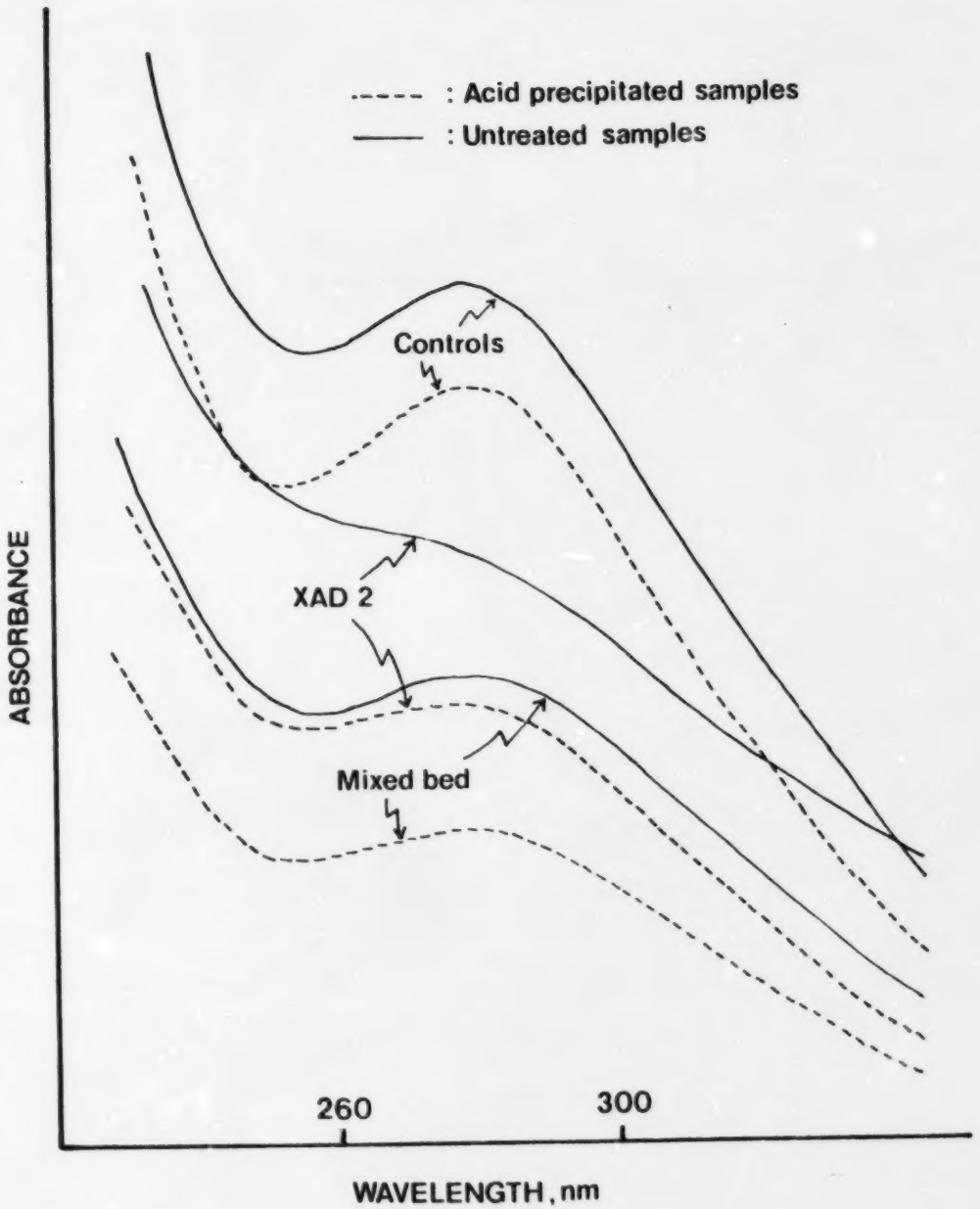


FIGURE 6 : The effect of acid and resin pretreatment on the absorbance profile of peat hydrolysate dilutions. (Final carbohydrate concentration = 0.06%).

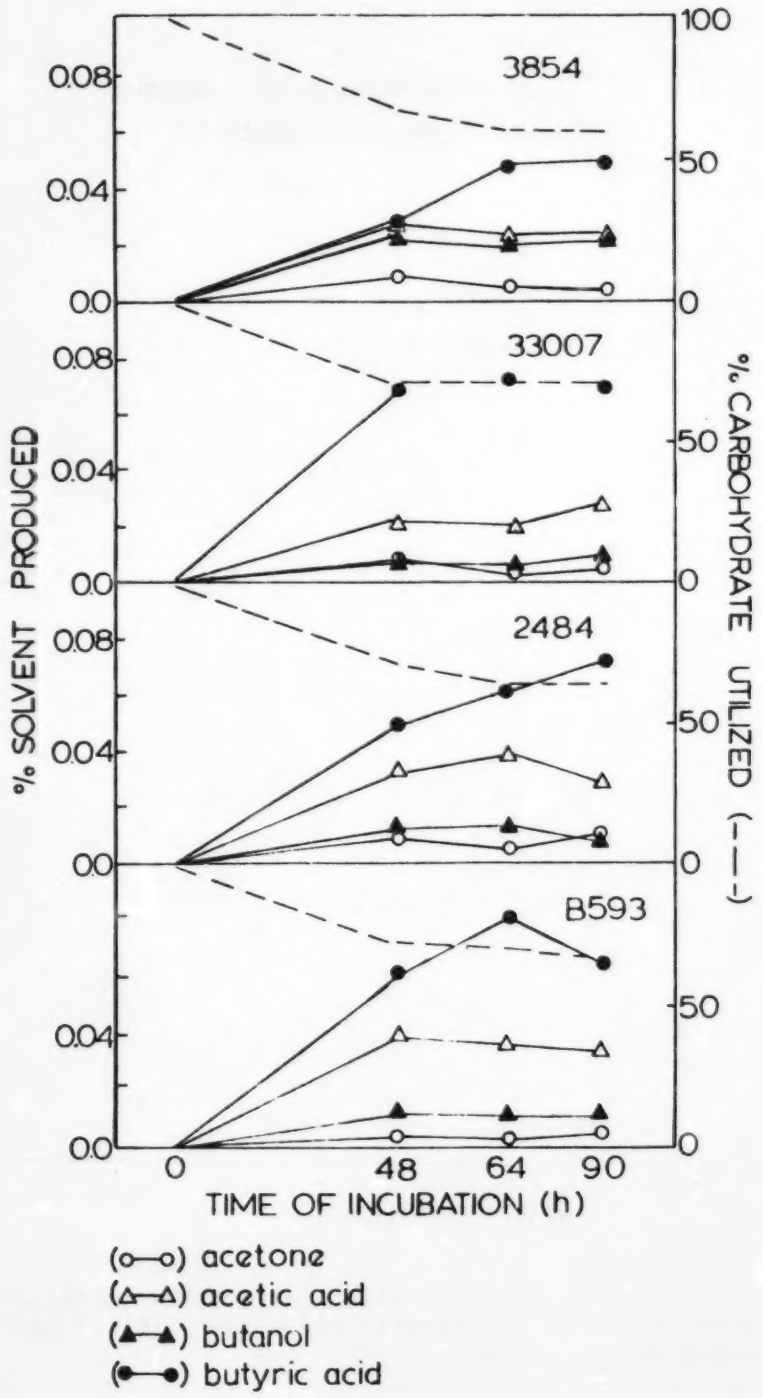


FIGURE 7 : Carbohydrate utilization and solvent production by Clostridium acetobutylicum 3854 and 2484 and Clostridium butylicum 33007 and B593 during growth in chemically defined medium containing 1.0% (wt/vol) peat extract carbohydrate.